10. During tetanisation of moderate strength and of whatever direction the normal current becomes positive (or less negative). This positive change gradually subsides.

11. Strong single induction shocks of whatever direction arouse prolonged positive after-effects, that gradually subside.

12. Single condenser discharges (2 to 10 M.F., 1 to 7 volts), of whatever direction, arouse prolonged positive after-effects.

13. In consequence of gentle massage of the eyeball, the normal current becomes strongly positive (or less negative). This positive change gradually subsides.

14. In consequence of gentle massage of the eyeball, the positive response of the 1st stage gives place to a negative response (*vide supra*, 5).

15. Fatigue—*i.e.*, diminution of response by reason of previous activity—is less pronounced in the case of the retina than in that of muscle. It is manifested in nearly the same degree to stimulation by light, and to stimulation by tetanising currents.

16. The positive response to light (2), the positive effect of tetanisation (10), and the positive after-effect of condenser discharges (13) are suppressed by anæsthetics (ether and chloroform) and by rise of temperature (to 40—45°). The suppression may be permanent or temporary. An anæsthetised like a dead eyeball tested by currents, as in 11 and 12, manifests only polarisation currents negative in direction to the exciting currents. Tetanisation, as in 10, gives only polarisation effect in the direction of the break shocks negative to the direction of the make shocks.

IV. Conclusions.

"Observations on the Effect of Desiccation of Albumin upon its Coagulability." By J. Bretland Farmer, M.A., Royal College of Science, London. Communicated by Dr. H. T. Brown, F.R.S. Received March 21,—Read April 5, 1900.

It has been known for some time that it is possible, under certain circumstances, to expose seeds to the influence of high temperatures without thereby necessarily destroying their power to germinate. Some experiments in this direction were conducted at the Royal Gardens, Kew, some years ago by Dr. Morris, but the results, although of much interest, do not appear to have been published. However, the seeds were exposed to the action of boiling water, and even to a higher temperature in an oven, without losing their ability to germinate when the ordeal was over.

It has been noticed, in heating seeds in water, that if the seed-coat

through any cause becomes ruptured, or if it softens and swells, the seeds which are thus affected are incapable of manifesting any further evidence of vitality. It appears to me that a fair inference to be drawn from these facts is that the admission of water to the living cells is a potent factor in bringing about their death.

Jodin* has recently communicated some facts which point to the same conclusion. He exposed seeds of pea and cress to a temperature of 98° C., and found that unless great care had been previously exercised to ensure the dryness of the seeds, they were all killed. When they had been previously dried he succeeded in subsequently germinating 30 per cent. of the peas and 60 per cent. of the cress seeds. Perhaps the disproportion in favour of the latter may, at least in part, be ascribed to their small size, and consequently to the less difficulty in sufficiently drying the seeds.

It would seem to follow from what has been said that the instability of the complex molecular structure of which living organisms are made up, may be lessened by appropriate desiccation, but the substances concerned are too complex to render themselves readily accessible to inquiry. It appeared, however, that it might be worth while to study the effects of desiccation on albumin from this point of view. Albumin is not only a highly complex proteid, and perhaps in some respects akin to protoplasm itself, but it is one which gives tolerably definite heat reactions. It is in connection with the last-mentioned point that the new facts in this paper are specially concerned.

It is of course known that albumin in a watery solution is readily coagulated on heating to a certain temperature. This temperature, however, is not necessarily constant for even one type of albumin, doubtless owing to the readiness with which it undergoes change. Thus albumin obtained from different hens' eggs will often be found to coagulate at different temperatures, and the differences appear, in part at any rate, to be connected with the age of the egg. I have found in the case of freshly-laid eggs, that the characteristic opalescence which marks the early stages of coagulation may set in as low as 60° C., the clotted coagulum being fully formed at 64° C. The heat was applied by means of a large water-bath, so as to ensure its being as uniform as possible. Another sample of albumin from a different egg tried simultaneously and under the same conditions, only exhibited opalescence at 65·6° C., and coagulated completely at 68° C.

The albumin on which most of my experiments were made, was obtained from Merck, of Darmstadt, and was sent as dried egg-albumin. It readily dissolved in water, with the exception of a little flaky insoluble portion, which was filtered off. The solution had a low coagulation-point, the opalescence appearing at 60° C., and the clot at 62° C.

* 'Comptes Rendus,' 1899.

to 63° C.* Filtering off the clot and testing the filtrate at higher temperatures yielded no further coagulation.

If a sample of this (dry) albumin be placed in a flask, the mouth of which is furnished with a cork and attached to a set of drying tubes, and the temperature of the flask raised to 80° C., a short exposure, of at any rate two to three hours, is enough to completely alter the albumin. The object of the drying tubes is to prevent any more moisture than is already present in its substance reaching the albumin from the steam or from any other source. Thus heated, the albumin is found to have become insoluble in water, and in fact to have undergone a change corresponding to coagulation.

If, however, the albumin be carefully *dried* before being subjected to these conditions, the results are quite different. For the present purpose it was found to be sufficient to expose a thin layer of albumin in a glass dish to a temperature of 52—55° C. in an incubator. This ensures a very thorough desiccation. The process may be hastened by introducing a vessel of sulphuric acid, though this precaution was not found to be necessary. Thus dried, the albumin loses its shellac or glue-like appearance, and easily crumbles to very small particles.

On comparing the solubility and coagulability of this specially dried material with the ordinary sample, no difference could be detected in any respect.

Numerous experiments were made with this dried material, of which the following may be taken as typical. It may be added that the results throughout were almost surprisingly uniform in the different experiments made.

A sample of the specially dried albumin was introduced in a flask so as to form a thin layer over the bottom. The flask was connected with drying tubes filled with calcium chloride, and with phosphorus pentoxide. The flask was warmed and cooled rapidly several times, in order to cause the contained air to circulate through the drying tubes.

The temperature of the flask was then raised in a brine bath to 102° C., and kept at this temperature during the whole of one day (six hours). Next day, and without opening or disturbing the apparatus, the temperature was again raised to 107° C., and finally to 110° C. It was maintained between these limits for seven hours; thus the contents of the flask had been for thirteen hours exposed to a temperature of considerably over 100° C.

On testing the albumin it was found to be soluble in water, and in no way, as far as could be observed, did it differ from the unheated material. On gradually warming the solution side by side with a

* It is of course known that several factors affect the coagulation point. The figures given represent those obtained in my experiments, which were all kept as uniform as possible so as to eliminate the factor of variability.

similar solution of the unheated albumin, both became opalescent at a temperature of 60° C., and both were completely coagulated at 62° C.

It thus appears that, if precautions are taken to ensure appropriate desiccation, it is possible to heat albumin for, at any rate, thirteen hours to a temperature varying between 102—110° C. without producing any obvious change in its ultimate molecular (or micellar ?) structure. It made no difference to the result whether the heat was gradually or rapidly applied. Thus, in one experiment, the temperature was raised from 50° C. to 103° C. in fifteen minutes, and in other examples the flask was withdrawn from the hot bath, cooled, and suddenly re-immersed. How much higher the temperature could be raised without producing an obvious effect, I am not prepared to say; nor did I investigate the action (if any) which might possibly be produced by a much longer exposure to heat within the limits already mentioned. This formed no part of my object, which was primarily to try to get a point of comparison between the complex seed and the simpler but still very complex proteid.

Other experiments were made in order to test the sensitiveness of the albumin to small quantities of moisture.

For this purpose, two flasks attached to drying tubes were used, one of them serving as a control experiment, and remaining unopened until the end. The other was opened three times, and a small sample taken out each time. By this means the ordinary air of the room obtained complete access to the albumin. The duration of the experiment was ten hours. The first sample was withdrawn after the flasks had been heated to 102° C. for three hours; it dissolved and coagulated normally. A second sample was withdrawn after three hours more, and it was found that whilst it dissolved and became opalescent on heating to 60° C., the coagulation change did not at once set in, but the opalescent solution became more milky and of a deeper fog-yellow by transmitted light, finally coagulating at about 68° C. A third sample taken out at the close of the experiment (*i.e.*, four hours after the last opening of the flask) also dissolved, became slightly opalescent at about 64° C., but did not coagulate even at 90° C., although the opalescent milkiness became very pronounced. Viewed by transmitted light, the solution was translucently yellow. Even boiling failed to produce anything which could be fairly termed a coagulum. It appeared probable that the admission of watery vapour had permitted the inception of the changes which normally, at high temperatures, result in coagulation; but in this case they were arrested, some precursor of alkali-albumin being probably produced, as is often the case on slowly coagulating albumin solutions. Under these circumstances, however, the entire mass of the albumin had undergone this change. This supposition turned out to be correct, for the addition of a trace of acetic acid at once caused the solution to

be susceptible to coagulation at about 60—62° C.* Hence it is fair to infer that although the slight amount of moisture introduced during the opening of the tube did not suffice to enable complete coagulation to occur, it did permit the early changes to begin, and to slowly, and in a modified way, to affect the entire mass. This experiment was repeated several times, and always with the same result.

It seems difficult, in the light of the foregoing observations, to resist the inference that in the complete absence of moisture albumin may be reduced to a state of relative molecular (or micellar) immobility; the rearrangements which, in the presence of water and at a sufficiently high temperature, normally take place in its ultimate structure being held in abeyance during the suspension of the essential condition of the presence of sufficient moisure. The substance is brought, so to speak, into a static condition; chemical or physico-chemical change is inhibited, just as is an interaction between phosphorus and oxygen when conditions of complete dryness obtain. It is tempting to extend these considerations to the case of seeds and spores, *e.g.*, of certain bacteria, and to ask whether similar conclusions may not be fairly assumed to obtain there, for it may well be a fact that the protoplasm, like the albumin, which is at any rate akin to it, when sufficiently desiccated withstands conditions which otherwise would certainly promote chemical disintegration. They, too, appear to be reduced to a "static" condition by drying, and the researches of Romanes† indicated no measurable chemical change as proceeding in them under these circumstances; and, again, the investigations of Brown and Escombe,‡ and of Sir W. Thiselton-Dyer,§ have also rendered it difficult to believe, when subjected to the other end of the scale of temperature, that any metabolism can really be proceeding. In these cases the molecular machinery of life is all present and intact, but the *manifestation of vitality*, as measured by chemical movement and by the change in the condition of energy, is absent. But such a state differs widely from death, seeing that when the conditions favourable to the continuous progress of those reactions which are associated with vitality are restored, the organism proceeds to work in the normal manner once more. Similarly the albumin heated in the desiccated form retains, instead of changing, that particular molecular condition which enables it, on restoring the essential conditions of moisture, to coagulate in a normal fashion when heated to a suitable degree of temperature.

* A solution of albumen treated with a very small quantity of a dilute solution of potash undergoes a similar change. The substance formed is not true alkali-albumen, since no precipitate is produced on neutralising, and a coagulum on heating this neutralised solution.

† 'Proc. Roy. Soc.,' vol. 57.

‡ *Ibid.*, vol. 62.

§ *Ibid.*, vol. 65.